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DYNAMICS OF RESISTANCE TO PLAGUE IN WHITE MICE AFTER A SINGLE INOCULATION OF FRACTION 1 OF THE PLAGUE MICROBE

[Following is the translation of an article by N. N. Basova and Yu. A. Filimonova, Rostov on the Don Antiplague Institute (Dir. - Prof. I. V. Domaradskiy), published in the Russian-language periodical Byull. Eksper. Biol. Med. (Bulletin of Experimental Biology and Medicine), 65(2), 1968, pages 83--86. (It was submitted on 25 May 1965 and presented by active member of the AMN USSR N. N. Zhukov-Verezhnikov.)]

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A single administration of fraction 1 of the plague microbe on complete Freund deponent 2 days prior to infection with a virulent culture of plague causative agent protected some of the white mice from death.

The wave-like (cyclic) nature of resistance to experimental plague and its intensity correlated with the dynamics of the antibodies detected in the serum.

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The mechanism of immunity in plague and the role of individual antigens in the creation of specific non-susceptibility have been studied intensively in many countries. Significant progress in this area has been achieved due to investigations which made it possible to establish the nature and value of the capsular substance, the so-called fraction 1 (F1). Proof has been obtained that the preventive properties of serum, revealed with the help of the test of passive protection of mice, are caused by the presence of antibodies to F1 in it. Resistance in animals which had been immunized or which had endured infection also correlates with the level of antibodies to F1 in their serum. It is doubtless that other antigens of the plague microbe take part in the formation of antibacterial and antitoxic immunity. However, a high content of antibodies only to fraction 1 is capable of ensuring resistance to large doses of virulent causative agent.

The aim of this work was a comparison of the dynamics of serological indices in white mice which had been inoculated one time with F1 with the dynamics of their resistance to experimental plague.

Experimental Methods

Purified preparation of F1 was obtained from the water-saline extract of a mass of bacteria by the Beyker method of salting out with ammonium sulfate (at 0.3--0.35 saturation) with subsequent multiple reprecipitation. This preparation produced one line of precipitation in gel against agglutinating antiplague serum. The activity of F1 in the antibody neutralization reaction (ANR) comprised 0.001 μg in 0.2 ml. The dose for immunization of one mouse equaled 20 μg, or 20,000 minimal neutralizing doses (MND). The antigen in a physiological solution of sodium chloride or in complete Freund deponent was administered into the underside of the rear extremities on the basis of 0.05 ml, containing 10 μg of F1, in each paw.

Serological investigations were conducted with the help of a stable diagnostic agent of high sensitivity - 2.5% suspension of formalinized tannin-treated sheep erythrocytes, charged with F1, in a 10% solution of formalin /1/. All the tests were set up with series of diagnostic agent of the same activity in respect to the agglutinating serum of the "Mikrob" Institute (series 69). The titer of this serum in the passive hemagglutination reaction (PHR) equaled 1:1,280,000.

The content of antigen and antibodies in the blood serum was determined in water-saline extracts of tissues from the site of administration of the antigen. After the taking of blood the mice were perfused with warm physiological solution (20--25 ml for each animal) through the major circulatory system. Severed paws were weighed and ground with a small amount of sand and a 10% solution was prepared. After centrifuging and heating at 56° for 30 minutes the water-saline extract was filtered through paper and absorbed by sheep erythrocytes. The sera which was absorbed by sheep erythrocytes and the treated extracts were tested in the PHA and the ANR. Specificity of the results obtained in the PHR was controlled with the help of the hemagglutination inhibition reaction (HIR) with 8--16 MND of antigen.

The resistance of the white mice was checked by infection with the virulent strain No 1300 of the plague causative agent. Uniformity of conditions of infection was ensured by the use of one generation of microbes, incubated on solid medium with casein hydrolyzate. After a 48-hour incubation at 28° the inoculation was stored in the cold for the entire test. Subcultures for infection were seeded off every 2 days onto solid medium of the same series. Tenfold dilutions of a 2-day culture of microbes were prepared on physiological solution. The number of live cells in the prepared suspensions was determined by the results of inoculation on plates. For infection the corresponding suspensions of microbes in a volume of 0.2 ml were administered subcutaneously to the white mice.

The white mice were immunized with F1 on complete Freund deponent and on physiological solution (350 and 200 animals respectively). Corresponding groups of animals (control) received deponent or physiological solution.

Results of experiments

Of the animals which were inoculated with F1 on deponent 3 mice were sacrificed each day. The content of antigen in their sera and extracts from tissues from the site of administration are shown in Figure 1. The curves are constructed on average geometric titers. In this test F1 ceased to be revealed in the serum of mice already in 2 days. On the 8th day antibodies were detected, the titer of which reached its highest index on the 10th day. But on the 11th and 12th days antibodies were not detected in the serum. On the 13th day they again started to be determined.

Figure 1.

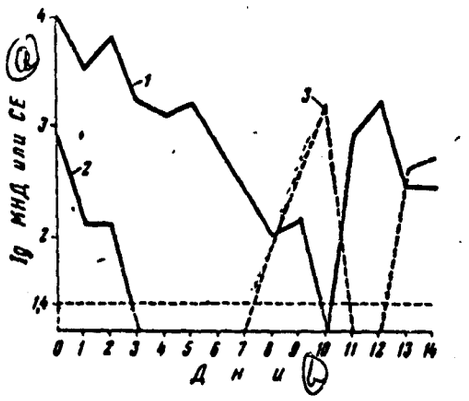

Figure 1. Dynamics in the content of antigen and antibodies in the blood and tissue extracts from the site of administration on mice which had been inoculated with F1 on deponent.
1 - curve of average concentrations of antigen in tissue extracts from the site of administration (MND - minimum neutralizing dose); 2 - the same in serum; 3 - curve of average concentrations of antibodies in the serum (SU - serum units). The horizontal dotted line is the level of reading.
Key: (a) lg of MND or SU; (b) days.

There is considerable interest in the dynamics of residence of antigen in the tissues from the site of administration. A high level of antigen in extracts was preserved for the first 6 days after immunization. The noticeable lowering of its concentration coincided with the time of appearance of antibodies in the serum. In the period of the highest level of antibodies in the serum the activity of tissue extracts from the site of administration in the MNR was reduced to 0. With the "disappearance" of antibodies from the serum there was a coincident "new" increase in the concentration

of antigen in tissue extracts from the site of administration. The antibodies which appeared in the serum on the 13th-14th day as if lowered the level of antigen at the site of administration.

The impression is created that in mice which have been immunized one time with capsular antigen of the plague microbe the dynamics of residence of antigen and antibodies in the blood and tissue extracts from the site of administration have a cyclic nature. We observed a similar regularity on other laboratory animals which were inoculated with F1 on deponent (white rats, guinea pigs, golden hamsters, and rabbits). A dependence was revealed between the species of animals, dose of antigen, and nature of the cycles - number of waves, their duration, and fluctuations in the level of antigen and antibodies. Statistically reliable results of tests, demonstrating the cyclic nature of the primary immunological reaction in white mice, are described in the literature /2/. The wave-like nature of the dynamics of antigen and antibodies clearly reflected the dynamics of a plasmocytic reaction.

Alternation of periods, when antigen is detected, with "waves" of free antibodies apparently takes place due to the depot state of the antigen. Initially its presence in the blood causes a "start-up" of the mechanism of formation of antibodies and a local reaction in the form of hyperemia and inflammation. The antibodies which appear form neutral complexes with the antigen which has been released from the deponent. Only with a surplus of one of the components - antigen or antibodies - is it possible to detect it. Periods when neither antigen nor antibodies are revealed possibly set in when there is an equivalence of their ratio in the organism and neutralization of antigen by antibodies.

The dynamics of resistance to experimental plague were studied in parallel on uniform groups of test (immunized) and control animals. The intervals between infections were short - 48—72 hours. In each period the mice were infected with ten-fold increasing doses - from 1 to 10,000 microbial cells (6 mice from a group for each dose). The results obtained were treated statistically. The indices of resistance are expressed in logarithms of LD_{50}, and standard deviations were calculated by the method of Ashmarin. In Fig. 2 it can be seen that the administration of 20 μg of F1 on physiological solution considerably increased resistance to infection and in a relatively late period - in 9—13 days after immunization. However, the same dose of antigen on deponent changed the sensitivity of the animals to a virulent causative agent. Already in 2 days after immunization the mice of the test groups turned out to be more protected than the control groups which received the deponent without antigen. Resistance increased up to the 11th day. On the 14th-16th day the resistance of immunized animals was reduced to the level at which the mice of the control groups were found. During a check of immunity in following periods a considerable resistance of immunized mice was again revealed which distinguished them considerably from the control.

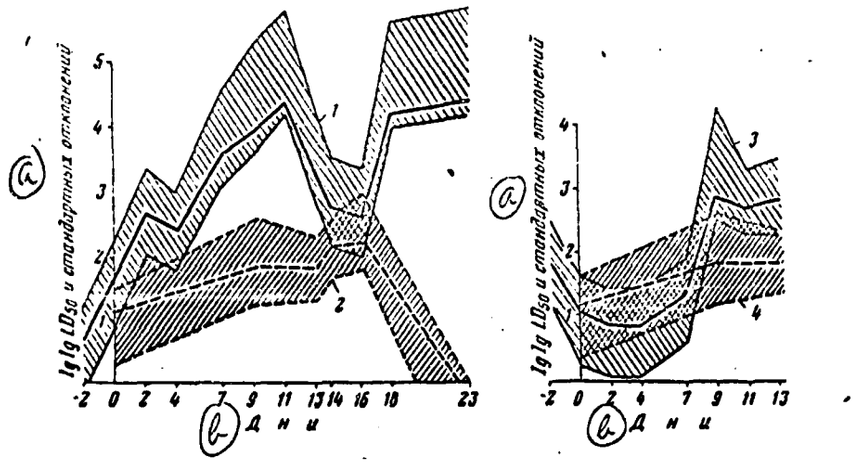

Figure 2. Dynamics of resistance to infection with virulent plague microbes.
1 - 4 - curve of lg LD_{50} and standard deviations (P = 0.95; processed by the formula of Kerber) respectively for white mice inoculated with deponent F1, mice which received only deponent, mice immunized with F1 on physiological solution, and control animals which received physiological solution. On the axis of abscissae - periods between immunization and infection (in days).
Key: (a) lg lg LD_{50} and standard deviations; (b) days.

A comparison of Fig. 1 and 2 shows that a bond exists between the curve of resistance and the dynamics of antigen and antibodies. The increase of resistance to infection by periods coincides with the periods of an increase in the titers of antibodies. The lowering of resistance, revealed on the 14th-16th day after administration of deponent F1, precedes the second wave of antibodies in the serum.

The early resistance observed shortly after administration of deponent F1 as if lacks an explanation: antibodies in detectable amounts were recorded only starting with the 8th day. Antibodies were not found in mice which had received F1 without deponent. This was checked with the help of serological reactions and the phagocytic test. It is very probable that in the early period after immunization the antibodies were not visible, the amount of them was not great, and they were used up rapidly, having become bound with the causative agent. The degree of resistance in the early periods was considerably lower than the protection of animals in later days, when it increased in parallel with an increase in the titer of antibodies. It was demonstrated that early protection in white mice, inoculated with F1, was specific: the animals resisted experimental plague, but not tularemia or melioidosis [3, 4].

Early developing resistance to foreign antigens has been described in cold-blooded animals and even insects. It is insufficiently specific and temporary. It can be assumed that the phenomenon of early protection was also preserved for more complexly organized creatures. As they become more developed the primary immunological reaction was specialized and became more specific.

Literature

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